Single and multiple topologically driven structural transitions in DNA

Igor Kulić*
Institut für Theoretische Physik 1, Universität Stuttgart, Pfaffenwaldring 57, D-70550 Stuttgart, Germany
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We derive some exact general results concerning the behavior of topological absorbers (i.e., sequences undergoing topologically driven structural transitions) in closed circular DNA molecules. Starting from the formal physical framework that covers all known structural transitions, like those from standard B-DNA to nonstandard conformers Z-DNA, H-DNA, cruciform-DNA, melt-DNA or others, we develop a reduced state space description that leads to an analytically simplified ''black box'' view of absorbers. The latter contains only a single state variable—the total sequence unwinding u describing the topological state of the absorber. We show that the statistical mechanics of u is determined by the (one-dimensional) absorption free energy function G_{abs} and find explicit expressions for G_{abs} and for moments $\langle u^n \rangle$ in terms of the standard experimental observable—the absorption function $\alpha := \langle u \rangle$. The reduced state space method is then applied to systems consisting of several interacting topologically coupled absorbers and a formula predicting their collective behavior (superposition) in terms of their individual absorptions is derived. Using these results we formulate and discuss solution methods for two basic types of inverse problems that turn out to be fundamental for future absorber construction.

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I. INTRODUCTION

Soon after the discovery of the DNA structure by Watson and Crick and especially since the beginning 1970s it increasingly became apparent by theoretical considerations and experimental studies that besides the importance of primary DNA structure (the sequence) its secondary and tertiary structure play a decisive biological role in all basic life processes.

Among these features two of them have been studied very extensively: alternative (non-B-DNA) conformations and the DNA topology [1] for their suspected relevance in replication [2], recombination [3] and transcription processes [4]. In the beginning of the 1980s the interplay between DNA topology and the occurrence of alternative non-B-DNA structures was discovered and studied both experimentally and theoretically (reviewed in [5,6]). Several alternative structures like cruciforms [7], melt DNA [8], and Z-DNA [9] have been studied in the context of supercoiled plasmids. In these studies it was clearly demonstrated that sequences undergoing a structural transition from standard B-DNA to alternative structures consume topological links available in the surrounding plasmid (which manifests as a reduction of overall supercoiling) and in fact do act like *topological absorbers* (see Fig. 1). It soon became apparent that the importance of topological absorbers is at least twofold. First their presence can change the level of supercoiling in the topological domain where they are placed (plasmid, for instance) and thereby affects strongly the global properties of the topological domain by local changes in only a single very short site (Fig. 1). The second important property of topological absorbers is their ability to perform fundamental biological regulatory tasks through their alternative conformations as parts of transcriptional or replicational initiation machinery either directly or by acting as specific binding sites for binding elements like transcriptional activators or for RNA polymerase.

The theoretical framework [5,7–9] for understanding the biophysics of topological absorbers can be summarized as follows. The supercoiling free energy of a plasmid of given length can be measured in dye intercalation and nicking-closing experiments [10]. From such experiments a simple law can be extracted: the free energy of a plasmid of total length N basepair (BP) containing λ additional negative links (manifested as supercoils) at temperature T can be described as

$$G_{sup}(\lambda) = \kappa R T \lambda^2, \tag{1}$$

with λ the negative topological excess linking number that is

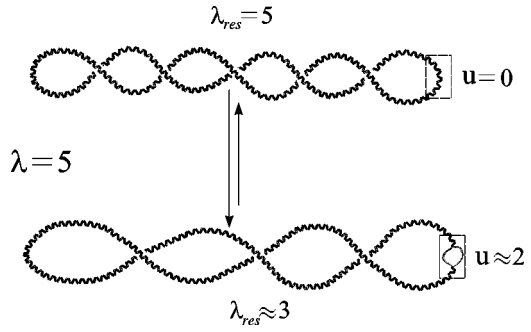

FIG. 1. A typical topologically driven local structural transition in a topologically closed negatively supercoiled plasmid. The topological absorber (sequence in the dashed box) undergoes a structural transition from the standard B-DNA form to the melted DNA structure and consumes $u \approx 2$ negative links. Because the number of total negative excess links λ is a topologically conserved quantity, the residual negative excess linking number $\lambda_{res} = \lambda - u$ must decrease during the transition from five to three links. The energetically unfavorable local DNA melting is compensated by a reduction of the supercoiling free energy ($G_{\mathrm{sup}} \propto \lambda_{res}^2$).

*Email address: igor@theo.physik.uni-stuttgart.de

imposed on the plasmid,[1] R the molar gas constant, and $\kappa = 1100/N$ a length dependent plasmid constant.

A topological absorber placed within a supercoiled plasmid will itself possess a free energy $G(s_1, s_2, \ldots, s_n)$ that depends on one or more inner variables $(s_1, s_2, \ldots, s_n) \in S$ that describe the structural state of the absorber in a state space S. The dimensionality of the underlying state space S and the concrete functional form of G will strongly depend on the type of the structural transitions the topological absorber is able to undergo, the base-sequence of the absorber (especially its translational, reflectional, and other symmetries), but also on the microscopic accuracy of the underlying theoretical model. This fact is well illustrated by the variety of models of different complexity for cruciform Z-DNA, melted DNA, or H-DNA forming absorbers that include one, two, or even up to $n = O(L)$ independent state variables, with L being the length of the underlying absorber in base pairs. For details we refer to the literature.

Now if we consider a composed system consisting of an absorber interacting with the rest of the plasmid it is possible to address a probability density to each state $\vec{s} := (s_1, s_2, \ldots, s_n) \in S$:

$$W_\lambda(\vec{s}) = \frac{1}{Q(\lambda)} e^{-[(1/RT)G(\vec{s}) + \kappa(\lambda - U(\vec{s}))^2]} \tag{2}$$

$$Q(\lambda) = \int_{\vec{s} \in S} e^{-[(1/RT)G(\vec{s}) + \kappa(\lambda - U(\vec{s}))^2]} ds_1 \ldots ds_n . \tag{3}$$

The norming factor in Eq. (2), the partition function $Q(\lambda)$ is defined by an n-dimensional integration/summation[2] over the whole state space S [Eq. (3)]. The topological *unwinding* U is a function of the state $\vec{s}$ that counts how many negative additional topological links are consumed by the absorber being in this state. Due to the famous link conservation law [1] the total excess links λ are shared among the two subsystems—the topological absorber sequence and the rest of the supercoiled molecule—but their total sum remains unchanged unless one of the DNA strands is broken. For that reason the additional links consumed by the absorber lead to a reduction of the links available in the rest of the plasmid (in the form of supercoils) to $\lambda - U(\vec{s})$; compare Figs. 1–3.

Having the probability density W_λ for each state $\vec{s}$ we can in principle compute the mean value of any function F on the state space:

$$\langle F \rangle(\lambda) = \int_{\vec{s} \in S} F(\vec{s}) W_\lambda(\vec{s}) ds_1 \ldots ds_n . \tag{4}$$

[1]We introduce $\lambda := -\triangle Lk$ for briefness, with $\triangle Lk$ being the number of excess links in the standard biological terminology.

[2]Depending on the absorber type and concrete theoretical modeling the state space may be either continuous or discrete in each of the dimensions $s_1, \ldots s_n$. Most results in this paper do not substantially depend on this distinction and we formulate them generally in terms of integrals.

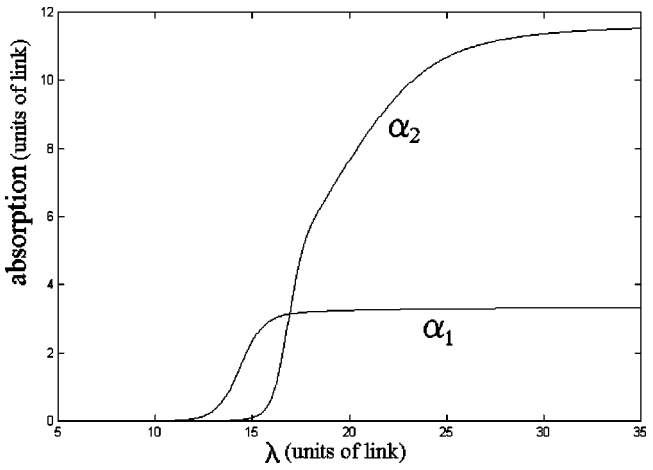

FIG. 2. The individual absorptions of two typical absorbers where α_1 corresponds to $(GC)_7$ and α_2 to $(GC)_{30}$ in a plasmid of $4kbp$ length at $T = 320$ K. Both curves are computed from the standard zipper model [9] for Z-DNA formation as developed in [9]. Their sigmoidal, strictly increasing shapes are representative for all single-absorber systems observed so far.

A very important function on the state space is the absorber unwinding function U itself. It turns out that its mean value —the *topological absorption function* (or simply the *absorption*) α,

$$\alpha(\lambda) := \langle U \rangle(\lambda) = \frac{1}{Q(\lambda)} \int_{\vec{s} \in S} U(\vec{s}) e^{-[(1/RT)G(\vec{s}) + \kappa(\lambda - U(\vec{s}))^2]} ds_1 \ldots ds_n , \tag{5}$$

can be easily measured in two-dimensional (2D) gel-electrophoresis experiments. Though not being the only experimentally available quantity describing the behavior of absorbers, the absorption α is the most commonly measured observable, and it has been obtained for all known absorber types in the past. As we will see later, α is in some way a

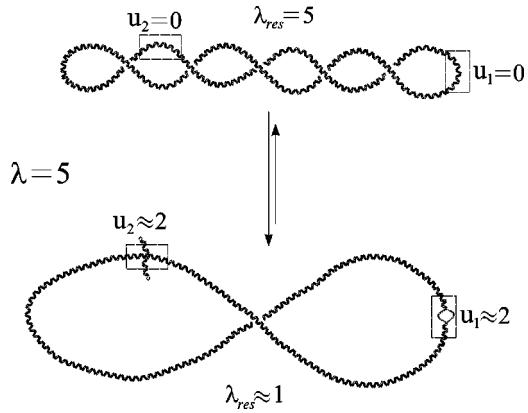

FIG. 3. Two distant topological absorbers compete for the available negative excess links in plasmid. The first of them, a melted DNA forming sequence, consumes u_1 negative links and the second one, a cruciform forming sequence, absorbs u_2. The driving force of the transition is again the reduction of G_{sup} (as in the single absorber case in Fig. 1) which leads to a strongly nonlocal, distance independent coupling between the two absorbers.

very fundamental quantity that allows a simplifying ''black box'' view of the absorber and also determines the absorber's interaction with other (topologically coupled) absorbers.

II. STATE SPACE REDUCTION AND THE BLACK BOX VIEW

Let us suppose we have measured the absorption α (as a function of the negative linking number difference λ of the plasmid) of an arbitrary (possibly unknown) absorber type that is placed in the context of a topologically closed plasmid for sufficiently many different values of λ. By idealizing we might assume α to be known on the whole real line.[3] What kind of information about the underlying absorber can we extract from α?

To answer this question we need to simplify analytically the state space of the underlying absorber. Therefore we reshape the expressions, Eq. (3) and Eq. (5), for the partition function Q and absorption α by a simple change of variables $(s_1,s_2\ldots,s_n)\rightarrow(u,s_2,\ldots,s_n)$,

$$Q(\lambda)=\int_{-\infty}^{\infty}e^{-\kappa(\lambda-u)^2}\times\left(\int_{\vec{s}\in S_u}\frac{1}{\left|\frac{\partial U(\vec{s})}{\partial s_1}\right|}e^{-(1/RT)G(\vec{s})}ds_2\ldots ds_n\right)du, \tag{6}$$

$$\alpha(\lambda)=\frac{1}{Q(\lambda)}\int_{-\infty}^{\infty}ue^{-\kappa(\lambda-u)^2}\times\left(\int_{\vec{s}\in S_u}\frac{1}{\left|\frac{\partial U(\vec{s})}{\partial s_1}\right|}e^{-(1/RT)G(\vec{s})}ds_2\ldots ds_n\right)du, \tag{7}$$

where the inner integration goes over $S_u:=\{\vec{s}\in S \text{ with } U(\vec{s})=u\}$, i.e., the $(n-1)$-dimensional surface in the state space with constant absorber unwinding (equal to u). We may now rewrite Eqs. (6) and (7) in a simpler way by introducing the *absorption free energy* G_{abs},

$$Q(\lambda)=\int_{-\infty}^{\infty}e^{-\kappa(\lambda-u)^2}e^{-(1/RT)G_{abs}(u)}du, \tag{8}$$

$$\alpha(\lambda)=\frac{1}{Q(\lambda)}\int_{-\infty}^{\infty}ue^{-\kappa(\lambda-u)^2}e^{-(1/RT)G_{abs}(u)}du, \tag{9}$$

with

$$G_{abs}(u):=-RT\times\ln\left(\int_{\vec{s}\in S_u}\frac{1}{\left|\frac{\partial U(\vec{s})}{\partial s_1}\right|}e^{-(1/RT)G(\vec{s})}ds_2\ldots ds_n\right). \tag{10}$$

The absorption free energy G_{abs} can be seen as an effective free energy of the absorber being in the topological unwinding state u (with an undetermined microscopic realization). G_{abs} is received by an integration/summation[4] over all states $\vec{s}$ that have the same total unwinding $U(\vec{s})=u$. u describes a collective state of all state variables s_i that has many inner microscopic realizations. Nevertheless, the variable u in such a reduced state space, together with $G_{abs}(u)$, is sufficient for describing the topological influence of the underlying absorber on the rest of the plasmid, as we will see below.

In the following and throughout our whole exposition, we will take the following simplifying point of view: we are not primarily interested in the microscopic details of the structural transition (which may well be interesting and important in some other context) and consider an absorber as being a ''black box'' that responds to different levels of topological stress (reflected in the negative linking difference λ) with a certain topological absorption α. From this point of view the absorption free energy G_{abs} becomes a central quantity because it allows us to abstract from the possibly sophisticated microscopic descriptions Eqs. (6) and (7) to obtain the relationship between λ and α, Eqs. (8) and (9). Therefore it is natural to ask the obvious question: If we can measure the absorption function α for sufficiently many points λ (or ideally for all real λ), can we compute the absorption free energy G_{abs} [Eq. (10)] from Eqs. (8) and (9)?

To answer this question we need to make several observations. First of all, the topological absorption α from Eq. (9) can be expressed more conveniently in terms of the partition function Q:

$$\alpha(\lambda)=\frac{1}{2\kappa}\frac{\partial}{\partial\lambda}\ln[e^{\kappa\lambda^2}Q(\lambda)], \tag{11}$$

which can be verified by simple computation. From Eq. (11) we compute Q:

$$Q(\lambda)=Q(0)\exp\left(2\kappa\int_0^{\lambda}\alpha(u)du-\kappa\lambda^2\right), \tag{12}$$

with an arbitrary positive constant $Q(0)$. Further we need to observe from Eq. (8) that Q is a Fourier convolution of two functions:

$$Q(\lambda)=[g*f](\lambda) \quad \text{with}$$

$$g(u):=e^{-(1/RT)G_{abs}(u)}, \tag{13}$$

[3]Although present experimental techniques with plasmids allow only rather modest resolutions (stepsize $\Delta\lambda=1$) and limited ranges for maximal and minimal values of λ, there seem to be no principle obstacles to overcoming these limitations in the future by significantly changing the experimental setup.

[4]In the case of discrete summation the term $|\partial U(\vec{s})/\partial s_1|$ (which stems from the functional deteriminant $\partial(s_1,s_2,\ldots s_n)/\partial(u,s_2,\ldots s_n)$) has to be dropped out.

$$f(u) := e^{-\kappa u^2}.$$

Finally, we factorize the convolution in Eq. (13) by applying the Fourier transform $\mathcal{F}$ and extract G_{abs} from $\mathcal{F}[g]$ by applying its inverse $\mathcal{F}^{-1}$. We obtain at last[5]:

$$G_{abs}(u) = -RT \ln\left\{ \mathcal{F}^{-1}\left[e^{\omega^2/4\kappa}\mathcal{F} \times\left[\exp\left(2\kappa \int_0^{\lambda} \alpha(t)dt - \kappa\lambda^2 \right)\right](\omega)\right](u)\right\} - C, \tag{14}$$

with

$$C := RT \ln\left(Q(0)\sqrt{\frac{\kappa}{\pi}}\right).$$

From the last equation we see that if the absorption function α of an arbitrary absorber is given we can determine its absorption free energy function G_{abs} (up to an arbitrary additive constant C). What cannot in general be deduced solely from the knowledge of α is the full free energy function $G(\vec{s})$ on the complete (nonreduced) state space S. This type of information goes beyond the simple observable α and must be obtained from additional assumptions on the exact structural transitions the absorber is able to undergo or from the measurement of further experimental observables that go beyond α.

Still α is a very useful observable and therefore we will examine in the next section what type of information about the equilibrium statistical mechanics of the underlying absorber we can extract from the knowledge of α only.

III. HIGHER MOMENTS AND CUMULANTS

As we noticed above, the absorption α of an absorber is (by definition) the first moment of its topological unwinding variable u. Here we want to compute the higher moments M_n of the random variable u in terms of the absorption α. To do this we must observe that for the moments,

$$M_n(\lambda) := \langle u^n \rangle(\lambda) = \frac{1}{Q(\lambda)} \int_{-\infty}^{\infty} u^n e^{-\kappa(\lambda-u)^2} e^{-(1/RT)G_{abs}(u)} du, \tag{15}$$

the following simply provable recursive relation holds:

$$M_{n+1}(\lambda) = \left(\frac{1}{2\kappa}\frac{\partial}{\partial\lambda} + \alpha(\lambda)\right) M_n(\lambda) \qquad \text{for} \quad n \geq 1. \tag{16}$$

Using $M_1 := \alpha$ we conclude from Eq. (16)

$$M_n(\lambda) = \left(\frac{1}{2\kappa}\frac{\partial}{\partial\lambda} + \alpha(\lambda)\right)^{n-1} \alpha(\lambda) \quad \text{for} \quad n \geq 1. \tag{17}$$

Based on this we can compute the first few moments:

$$M_1 = \alpha,$$

$$M_2 = \alpha^2 + \frac{1}{2\kappa}\alpha',$$

$$M_3 = \alpha^3 + \frac{3}{2\kappa}\alpha\alpha' + \frac{1}{(2\kappa)^2}\alpha'', \tag{18}$$

$$M_4 = \alpha^4 + \frac{3}{(2\kappa)^2}(\alpha')^2 + \frac{4}{(2\kappa)^2}\alpha\alpha'' + \frac{6}{2\kappa}\alpha^2\alpha' + \frac{1}{(2\kappa)^3}\alpha'''.$$

As we can see, the moments M_n are combinations of derivatives of α and their powers. The characteristic plasmid parameter κ and its powers also enter the expansion.

Although Eq. (17) allows effective computations of higher moments, it is interesting to express the moments M_n in a different way. To do so we consider the probability density of the unwinding variable u,

$$W_{\lambda}(u) := \frac{e^{-[(1/RT)G_{abs}(u) + \kappa(\lambda-u)^2]}}{Q(\lambda)} \tag{19}$$

for which we obviously [Eqs. (9) and (8)] have

$$\alpha(\lambda) = \int_{-\infty}^{\infty} u W_{\lambda}(u) du. \tag{20}$$

On the other hand, we can substitute Eq. (8) in Eq. (11) and rewrite the expression slightly by introducing an additional (independent) variable t:

$$\alpha(\lambda) = \frac{1}{2\kappa}\frac{\partial}{\partial\lambda} \ln\left[\int_{-\infty}^{\infty} W_t(u) e^{2\kappa(\lambda-t)u} du\right]. \tag{21}$$

Now, it is interesting to note that the expression Eq. (21) can be interpreted in terms of the generating function ψ_t of the density function W_t, which is generally defined as

$$\psi_t(\omega) := \langle e^{\omega u} \rangle_{W_t} = \int_{-\infty}^{\infty} W_t(u) e^{\omega u} du, \tag{22}$$

where $\langle\ \rangle_{W_t}$ means the mean in regard to the density W_t. Using this, α can be rewritten as

$$\alpha(\lambda) = \frac{1}{2\kappa}\frac{\partial}{\partial\lambda} \ln \psi_t[2\kappa(\lambda - t)] = \frac{1}{2\kappa}\frac{\partial}{\partial\lambda} C_t[2\kappa(\lambda - t)], \tag{23}$$

where $C_t := \ln \psi_t$ is the cumulant generating function of W_t. The last expression in Eq. (23) allows us to relate the derivatives of the absorption α with the sequence of cumulants $c_{t,n}$ belonging to the corresponding probability density W_t. After

[5] ω is the variable in the Fourier space.

integration over λ of Eq. (23) followed by Taylor expansion of both sides we receive

$$c_{t,n}=\frac{1}{(2\kappa)^{n-1}}\alpha^{(n-1)}(t) \quad \text{for} \quad n\geqslant 1 \tag{24}$$

with $c_{t,n}$ being the nth cumulant of W_t. Having this we can use the well-known relationship between the cumulants $c_{t,n}$ and the moments $M_n(t)$ (see [17]) to express the moments in terms of the absorption derivatives $\alpha^{(n)}(t)$:

$$M_n=\det\begin{pmatrix} \alpha & -1 & 0 & 0 & 0 & \dots \\ \frac{1}{2\kappa}\alpha' & \alpha & -1 & 0 & 0 & \ddots \\ \frac{1}{(2\kappa)^2}\alpha'' & \frac{1}{2\kappa}\binom{2}{1}\alpha' & \alpha & -1 & 0 & \ddots \\ \frac{1}{(2\kappa)^3}\alpha^{(3)} & \frac{1}{(2\kappa)^2}\binom{3}{1}\alpha'' & \frac{1}{2\kappa}\binom{3}{2}\alpha' & \alpha & -1 & \ddots \\ \frac{1}{(2\kappa)^4}\alpha^{(4)} & \frac{1}{(2\kappa)^3}\binom{4}{1}\alpha^{(3)} & \frac{1}{(2\kappa)^2}\binom{4}{2}\alpha'' & \frac{1}{2\kappa}\binom{4}{3}\alpha' & \alpha & \ddots \\ \dots & \dots & \dots & \dots & \dots & \ddots \end{pmatrix}_n . \tag{25}$$

If we want to express $\alpha^{(n)}$ in terms of the moments M_n a similarly elegant formula holds:

$$\alpha^{(n)}=(-2\kappa)^n\det\begin{pmatrix} M_1 & 1 & 0 & 0 & 0 & \dots \\ M_2 & M_1 & 1 & 0 & 0 & \ddots \\ M_3 & M_2 & \binom{2}{1}M_1 & 1 & 0 & \ddots \\ M_4 & M_3 & \binom{3}{1}M_2 & \binom{3}{2}M_1 & 1 & \ddots \\ M_5 & M_4 & \binom{4}{1}M_3 & \binom{4}{2}M_2 & \binom{4}{3}M_1 & \ddots \\ \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}_{n+1} . \tag{26}$$

IV. SOME IMPLICATIONS

There are several implications that follow from the moment equations stated above. They concern the transition behavior of absorbers and the shape of their absorptions α. The first geometrical implication follows from centered moments $V_n(\lambda):=\langle(u-\alpha)^n\rangle_{W_\lambda}$, the first of which are

$$\begin{aligned} V_0&=V_1=0, \\ V_2&=\frac{1}{2\kappa}\alpha', \\ V_3&=\frac{1}{(2\kappa)^2}\alpha'', \\ V_4&=\frac{1}{(2\kappa)^2}\alpha'''+\frac{3}{(2\kappa)^2}(\alpha')^2. \end{aligned} \tag{27}$$

From the second line we can compute the dispersion

$$\sigma:=\sqrt{V_2}=\sqrt{\frac{1}{2\kappa}\alpha'}. \tag{28}$$

Keeping in mind that the plasmid constant κ and the variance V_2 are positive quantities we conclude that α must be an increasing function of λ, that is,

$$\alpha'(\lambda)>0. \tag{29}$$

This is the first universal geometric property of absorptions of single absorbers.[6] It is remarkable that this property was observed in all experiments with single absorbers ([7–9]), but did not get much theoretical attention in the past. Its importance lies in the fact that it can only be violated in systems of two or more coupled absorbers ([14–16], see Fig.

[6]In systems consisting of several interacting absorbers this property is sometimes violated ([14,15], Fig. 4). The criteria for the occurrence of such anomalous effects will be discussed in depth elsewhere [18].

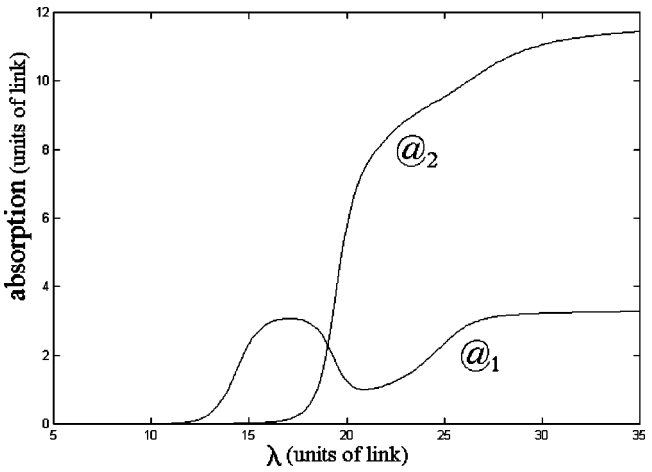

FIG. 4. Conditional absorptions of the two absorbers from Fig. 2. The anomalous effect of decreasing absorptions ($d@_1/d\lambda<0$) occurs exclusively in systems consisting of two or more absorbers. This feature can be used to distinguish between single- and multiple-absorber systems.

4), and therefore can be used as an indicator for the presence of other absorbers in the same topological region. More precisely: if it happens that $\alpha'(\lambda)<0$ for some λ, then we certainly know that the absorber is not alone in the plasmid and that it interferes with some other hidden or unknown absorber(s) in the same plasmid. From higher centered moments we can derive further geometric restrictions on the shape of α that can also be used as indicators for hidden additional absorbers.

Having the lower boundary ($=0$) for α' we may ask the opposite question: is there an upper boundary for α', or equivalently, can the structural transition of an absorber be arbitrarily ''sharp'' (in terms of the variable λ)? We can easily answer this in the case when the absorber is state limited. By a *state-limited* absorber we simply mean an absorber whose absorption free energy G_{abs} is finite only in a limited interval and infinite outside this interval, i.e. for its absorption free energy we assume

$$G_{abs}(u)=\begin{cases}<\infty & \text{for } u\in(u_{\min},u_{\max}),\\ \infty & \text{otherwise.}\end{cases} \tag{30}$$

For that free energy we have that the probability density $W_\lambda(u)$ [Eq. (19)] vanishes outside the interval $(u_{\min},u_{\max})$. In this case, which is much less restrictive than it might seem at the first glance,[7] we can perform the following estimation:

$$u_{\min}\leqslant\alpha(\lambda)\leqslant u_{\max}, \tag{31}$$

which implies

$$\begin{aligned}\frac{1}{2\kappa}\alpha'(\lambda)&=V_2(\lambda)\\&=\frac{\int_{u_{\min}}^{u_{\max}}[u-\alpha(\lambda)]^2e^{-(1/RT)[G_{abs}(u)+G_{\sup}(\lambda-u)]}du}{\int_{u_{\min}}^{u_{\max}}e^{-(1/RT)[G_{abs}(u)+G_{\sup}(\lambda-u)]}du}\\&\leqslant\frac{\int_{u_{\min}}^{u_{\max}}(u_{\max}-u_{\min})^2e^{-(1/RT)[G_{abs}(u)+G_{\sup}(\lambda-u)]}du}{\int_{u_{\min}}^{u_{\max}}e^{-(1/RT)[G_{abs}(u)+G_{\sup}(\lambda-u)]}du}\\&=(u_{\max}-u_{\min})^2,\end{aligned}$$

$$0<\alpha'(\lambda)\leqslant 2\kappa(u_{\max}-u_{\min})^2. \tag{32}$$

The meaning of the last estimation can be summarized as follows: the slope of the absorption α of a state-limited absorber is limited by the square of the difference of the maximal and the minimal unwinding state ($u_{\max}$ and $u_{\min}$) and the plasmid constant κ. The two extremal states can be easily extracted from the shape of α and are obtained from

$$u_{\max/\min}=\lim_{\lambda\to\pm\infty}\alpha(\lambda).$$

The plasmid constant κ is itself inversely proportional to the length N of the plasmid ($\kappa=1100/N$), so that the estimation (32) is more restrictive for longer plasmids. This is consistent with the common experimental observation that structural transitions of absorbers in shorter plasmids are sharper (α' may be higher) than in longer ones.

V. SUPERPOSITION LAW FOR SEVERAL INTERACTING ABSORBERS

In previous sections we have considered the case of a single absorber being alone in a topologically closed plasmid, i.e. there was no interference with other absorbers in the same region. Here we want to discuss the case when two or more different absorbers $\mathcal{A}_1,\mathcal{A}_2\ldots$ are topologically coupled and strongly interact with each other by competing for available links in the plasmid where they are placed (Fig. 3). In order to describe this situation we need to formally distinguish between different types of absorptions. The single absorber absorptions (i.e., the ''interaction free'' absorptions) of different absorbers $\mathcal{A}_i$ we considered previously, we will abbreviate with α_i and call *unconditional* absorptions. Each of them results from the underlying absorption free energy $G_{abs,i}$ of the absorber $\mathcal{A}_i$ in the same way as we have had in Eq. (9):

$$\alpha_i(\lambda)=\frac{1}{Q_i(\lambda)}\int_{-\infty}^{\infty}u_ie^{-[(1/RT)G_{abs,i}(u_i)+\kappa(\lambda-u_i)^2]}du_i \tag{33}$$

$$Q_i(\lambda)=\int_{-\infty}^{\infty}e^{-[(1/RT)G_{abs,i}(u_i)+\kappa(\lambda-u_i)^2]}du_i. \tag{34}$$

[7] In fact, several natural absorber types like Z-DNA and cruciform DNA forming sequences are standardly modeled as state-limited absorbers.

On the other hand, if the absorbers $\mathcal{A}_i$ interact with each other, their primordial unconditional absorptions α_i will interfere and we will measure new—*conditional* absorptions (Fig. 4). The conditional absorption of an absorber $\mathcal{A}_k$ that interacts with the set of other absorbers $\{\mathcal{A}_1, \ldots, \mathcal{A}_{k-1}, \mathcal{A}_{k+1}, \ldots, \mathcal{A}_n\}$ we will from now on abbreviate with $@_k$. It can be shown (see Appendix B) that the conditional absorptions $@_k$ can be expressed exclusively in terms of the absorption free energies $G_{abs,i}$ of the absorbers taking part in the interaction. The inner state variables and the ''fine structure'' of each of the absorbers [reflected in their complete free energies $G_i(\vec{s})$ as introduced in the full state space description in Eq. (2) and Eq. (3)] turn out *not* to be crucial for their interaction as long as $G_{abs,i}$ is known. The total unwinding variables u_k (but not the variety of inner variables of each absorber) govern the topological interaction so that the conditional absorptions are written as

$$@_k(\lambda) = \frac{\int_{-\infty}^{\infty} \ldots \int_{-\infty}^{\infty} u_k \exp - \left[\frac{1}{RT} \sum_{i=1}^{n} G_{abs,i}(u_i) + \kappa \left(\lambda - \sum_{i=1}^{n} u_i \right)^2 \right] du_1 \ldots du_n}{Q(\lambda)}, \tag{35}$$

$$Q(\lambda) = \int_{-\infty}^{\infty} \ldots \int_{-\infty}^{\infty} \exp - \left[\frac{1}{RT} \sum_{i=1}^{n} G_{abs,i}(u_i) + \kappa \left(\lambda - \sum_{i=1}^{n} u_i \right)^2 \right] du_1 \ldots du_n . \tag{36}$$

Obviously the conditional absorption $@_k = \langle u_k \rangle$ is again the mean value of the unwinding variable u_k of the absorber $\mathcal{A}_k$ as we have had in the noninteracting absorber case [Eqs. (33),(34)] but in order to obtain $@_k$ (due to the coupling) it is not sufficient to know only the free energy function of $\mathcal{A}_k$ itself—$@_k$ is a collective quantity that results from the complete set of absorption free energies $G_{abs,i}$ of all the interacting absorbers in the system. Despite this complication, it is possible to compute a conditional absorption $@_k$ from the set of unconditional absorptions $\{\alpha_i\}_{i=1 \ldots n}$ by exploiting the relationship Eq. (14) and by substituting the absorption free energies obtained there (as functionals of α_i) in Eq. (35) and Eq. (36). Consequently the conditional absorptions $@_k = @_k[\alpha_1, \ldots, \alpha_n]$ can be viewed as functionals of the set of unconditional ones. Unfortunately, due to the complexity of Eqs. (14), (35), and (36), this relationship is analytically not very transparent. But if we consider

$$\begin{aligned} S[\alpha_1, \ldots, \alpha_n](\lambda) &:= \sum_{i=1}^{n} @_i(\lambda) \\ &= \frac{\int_{-\infty}^{\infty} \ldots \int_{-\infty}^{\infty} \left(\sum_{i=1}^{n} u_i \right) \exp - \left[\frac{1}{RT} \sum_{i=1}^{n} G_{abs,i}(u_i) + \kappa \left(\lambda - \sum_{i=1}^{n} u_i \right)^2 \right] du_1 \ldots du_n}{Q(\lambda)}, \end{aligned} \tag{37}$$

the sum of all conditional absorptions in the system, a quantity that we will call the *superposition* of $\{\alpha_i\}_{i=1 \ldots n}$, we can discover a much simpler analytical relationship. To do so we need to notice that the superposition S obeys the same identities as any unconditional absorption does, especially that Eq. (11) still holds

$$S[\alpha_1, \ldots, \alpha_n](\lambda) = \frac{1}{2\kappa} \frac{\partial}{\partial \lambda} \ln[e^{\kappa \lambda^2} Q(\lambda)], \tag{38}$$

with Q defined in Eq. (36). This analogy with Eq. (11) is surprising only at the first glance, as we may look at the many absorber system consisting of absorbers $\{\mathcal{A}_1 \ldots \mathcal{A}_n\}$ as a ''big composed'' absorber whose unconditional absorption is simply $S[\alpha_1, \ldots, \alpha_n]$. On the one hand we can extract Q from Eq. (38) as we did in Eq. (12), and on the other hand we may write Q as a convolution of $n+1$ functions [similarly to Eq. (13)]:

$$Q(\lambda) = (f_1 * \ldots * f_n * g)(\lambda) \tag{39}$$

with

$$f_i(u) := e^{-(1/RT) G_{abs,i}(u)},$$

$$g(u) := e^{-\kappa u^2}.$$

As in the second section, we can now combine Eqs. (38) and (39) and apply the Fourier transform to receive

$$\prod_{i=1}^{n} \mathcal{F}[f_i] = \frac{Q(0)}{\mathcal{F}[g]} \mathcal{F} \times \left[\exp \left(2\kappa \int_0^{\lambda} S[\alpha_1, \ldots, \alpha_n](u) du - \kappa \lambda^2 \right) \right]. \tag{40}$$

On the other hand, we can perform the same transforms for each absorber $\mathcal{A}_i$ (with unconditional absorption α_i) separately and get

$$\mathcal{F}[f_i] = \frac{Q_i(0)}{\mathcal{F}[g]} \mathcal{F} \left[\exp \left(2\kappa \int_0^{\lambda} \alpha_i(u) du - \kappa \lambda^2 \right) \right]. \tag{41}$$

Comparing Eqs. (41) and (40) we see that by introducing an appropriate transformation we can factorize the superposition operator S in terms of its arguments:

$$\chi[S[\alpha_1,\ldots,\alpha_n]]=\frac{\prod_{i=1}^{n} Q_i(0)}{Q(0)}\prod_{i=1}^{n}\chi[\alpha_i], \tag{42}$$

with the transformation χ (of a function φ) given by

$$\chi[\varphi](\omega):=\sqrt{\frac{\pi}{\kappa}}\exp[(1/4)\kappa\omega^2]\times\mathcal{F}\left[\exp\left(2\kappa\int_0^{\lambda}\varphi(u)du-\kappa\lambda^2\right)\right](\omega). \tag{43}$$

The transformation χ possesses a well defined inverse χ^{-1} that can be easily calculated,

$$\chi^{-1}[\psi](\lambda):=\frac{1}{2\kappa}\frac{\partial}{\partial\lambda}\{\ln\mathcal{F}^{-1}[\psi(\omega)e^{-(1/4\kappa)\omega^2}](\lambda)\}+\lambda. \tag{44}$$

If we look at Eq. (44) we observe that χ^{-1} is insensitive to factors multiplying its argument ψ, i.e., $\chi^{-1}[\text{const}\times\psi]\equiv\chi^{-1}[\psi]$. Due to that fact we can rewrite Eq. (42) in terms of χ^{-1} and eliminate the constant $[\Pi_{i=1}^{n}Q_i(0)]/Q(0)$ to receive the final form of the superposition law:

$$S[\alpha_1,\ldots,\alpha_n]=\chi^{-1}\left[\prod_{i=1}^{n}\chi[\alpha_i]\right]. \tag{45}$$

From this identity we can directly compute the superposition of n arbitrary absorbers $\{\mathcal{A}_i\}_{i=1..n}$ from their unconditional absorptions $\{\alpha_i\}_{i=1..n}$. Moreover, with Eq. (45) we can solve some very interesting biologically motivated inverse problems, as we will see in the next section.

VI. INVERSE PROBLEMS OF ABSORBER DESIGN

Absorbers are suspected to act as controlling elements in many genes either directly as binding regions for components of the transcription machinery or indirectly by (topologically) interfering with the conformational states of such regions. Changing the properties of absorbers or introducing new absorbers is therefore a possible method to target gene functionality and regulative behavior ([19]). There are two kinds of relevant inverse problems (IPs) that occur in this context:

IP 1. Suppose that in a DNA region there is a naturally occurring absorber $\mathcal{A}_1$ that performs some specific (indirect) regulative task by responding to different levels of topological links λ with different average unwinding (i.e., unconditional absorption) $\alpha_1(\lambda)$. Now we want to artificially introduce a second absorber $\mathcal{A}_2$ (with some absorption α_2) that interferes with $\mathcal{A}_1$ in such a way that their superposition $S[\alpha_1,\alpha_2]$ has a predefined shape and behavior as a function of λ. How can we compute the desired α_2 in terms of α_1 and $S[\alpha_1,\alpha_2]$?

IP 2. We have the same situation as in IP 1, i.e., we have a given unconditional absorption α_1 of an absorber $\mathcal{A}_1$ and we introduce a second absorber $\mathcal{A}_2$ with α_2, but now we demand a predefined form for the conditional absorption $@_1$ (instead of S). How do we need to choose α_2 in order to obtain the desired $@_1$?

Using the superposition law Eq. (45) from the previous section it is easy to solve the first problem IP 1 by applying the transform χ and its inverse [Eqs. (43),(44)]:

$$\alpha_2=\chi^{-1}\left[\frac{\chi[S[\alpha_1,\alpha_2]]}{\chi[\alpha_1]}\right]. \tag{46}$$

Such an absorber with unconditional absorption α_2 always exists if $\chi[S[\alpha_1,\alpha_2]]/\chi[\alpha_1]$ is *positive definite*.[8]

The solution of IP 2 (given α_1 and $@_1$, compute α_2) is less straightforward, and needs to be computed from a Fredholm-type integral equation (of the first or second kind). To obtain this equation we need to observe that the conditional absorption $@_1$ can also be rewritten as

$$@_1=\frac{f_2*(\alpha_1 Q_1)}{f_2*Q_1}, \tag{47}$$

with

$$Q_1(\lambda)=Q_1(0)\exp\left(2\kappa\int_0^{\lambda}\alpha_1(u)du-\kappa\lambda^2\right)$$

and

$$f_2(u)=\exp\left(-\frac{1}{RT}G_{abs,2}(u)\right).$$

We may now rewrite Eq. (47) to receive the Fredholm equation of the first kind,

$$\int_{-\infty}^{\infty}K(\lambda,u)f_2(u)du=0 \tag{48}$$

with

$$K(\lambda,u):=[@_1(\lambda)-\alpha_1(\lambda-u)]Q_1(\lambda-u).$$

All solutions f_2 of Eq. (48) that obey the conditions $f_2\geqslant 0$ and $\int_{-\infty}^{\infty}f_2<\infty$ lead to $G_{abs,2}$, and by applying Eqs. (9) and (8) we obtain the desired absorption α_2 that solves our inverse problem of type 2 for given $@_1$ and α_1.

There is also another equivalent formulation of Eq. (48) that may be more convenient for iterative solutions of IP 2. It can be obtained from Eq. (47) by reshaping and applying the Fourier transform:

$$(\mathcal{F}[f_2]\mathcal{F}[Q_1])*\mathcal{F}[@_1]=\mathcal{F}[\alpha_1 Q_1]\mathcal{F}[f_2],$$

which is a Fredholm integral equation of the second kind:

$$\int_{-\infty}^{\infty}\tilde{K}(s,t)\chi_2(t)dt=\chi_2(s), \tag{49}$$

with

[8] f is said to be positive definite if there is some g with $g\geqslant 0$ and $\int_{-\infty}^{\infty}g(x)dx<\infty$ so that $f=\mathcal{F}(g)$ holds.

$$\tilde{K}(s,t) := \frac{\mathcal{F}[Q_1](t) \cdot \mathcal{F}[@_1](s-t)}{\mathcal{F}[\alpha_1 Q_1](s)}$$

and

$$\chi_2 := \mathcal{F}[f_2].$$

Once we compute the solution χ_2 of Eq. (49) we can obtain α_2 simply by applying the transform χ^{-1} on χ_2.

VII. CONCLUSION

We have seen that much of the theory of sequences undergoing structural transitions in topologically closed DNA molecules (which we named topological absorbers) can be developed in a natural way from the knowledge of a simple but fundamental observable—the absorption function α. We have shown that every absorber can be brought to a reduced form in which its transitional behavior is described by only a single state variable—the total topological sequence unwinding u and the corresponding free energy G_{abs}, which is uniquely determined by the absorption function α [Eq. (14)]. Within this reduced state space description we neglect detailed structural information about the different conformational realizations corresponding to an unwinding u. This kind of view that can be termed with ''black box'' view contains of course less information than the full description but also its focusing lies elsewhere. If we are only interested in how a given absorber topologically responds to different levels of supercoiling (given by λ) and how it thereby topologically affects its surroundings, the black box view means an appropriate analytical simplification of the problem. It becomes especially useful in those systems for which our knowledge about the microscopic details of the underlying structural transition is still incomplete; for instance, in the case of sequences transforming from B-DNA to H-DNA [16] and other similarly complex absorbers.

Furthermore, we have seen that besides G_{abs} the absorption α determines explicitly all relevant statistical features of the random variable u (topological unwinding of the absorber), and we have derived explicit formulas for the moments M_n and cumulants c_n of u that depend on α and drawn some interesting conclusions about the shape of α. The most remarkable conclusion is that $\partial\alpha/\partial\lambda > 0$, i.e., α is a nondecreasing function of the total number of additional negative links λ in the topologically closed plasmid. Consequently, if for some absorber we should experimentally observe $\partial\alpha/\partial\lambda < 0$, we may conclude that the considered absorber is not alone in the plasmid. That means that there must be an additional, possibly unidentified (hidden), absorber in the same region that topologically interacts with the first absorber.

Further we have treated general systems of topologically coupled absorbers that have already been examined in a number of theoretic and experimental studies in the past [14,15]. We have observed (see Appendix B) that for understanding the topological interaction between absorbers it is sufficient to work within the reduced state space model, i.e., the black box view, and provided that we know the absorption free energies $G_{abs,i}$ we can predict the individual as well as the collective behavior of an arbitrary number of topologically coupled absorbers.

To distinguish conceptually between the behavior of uncoupled and coupled absorbers we have introduced the two different concepts: the unconditional and conditional absorptions. In the following we have considered the sum of all conditional absorptions in a topological domain, which we termed superposition, and we have found an explicit formula [Eq. (45)] that relates the unconditional absorptions $\{\alpha_i\}_{i=1\ldots n}$ with their superposition $S[\alpha_1, \ldots, \alpha_n]$ by applying a factorizing transform χ [Eq. (43)]. We have seen that it is possible to exploit the latter relationship to solve an interesting inverse problem (IP 1) that will naturally arise in future attempts to experimentally construct absorbers of desired behavior, i.e., of predefined absorptions. We have also considered a second even more important type of inverse problems, IP 2, and have transformed it to a form in which it can be subjected to standard numerical solution methods. We suspect that all relevant inverse problems of future absorber design can be split into a combination of these two basic inverse problems, IP 1 and IP 2. Keeping in mind the well established and significant importance of topological absorbers for basic genetic processes, we conclude that it is of great interest to know more about the principal physical solubility of these inverse problems in concrete situations.

The exploration of such questions and their practical implementation, especially in the context of gene behavior modulation in living systems, remain basic challenges for future research.

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APPENDIX A: AN EXAMPLE FOR STATE SPACE REDUCTION

In previous sections we have assumed the state space to be continuous, and for computing the basic quantities like Q, α, M_n, and others we applied integration over the whole state space. In is easy to see that the same formulas apply if we are dealing with a discrete state space. In this case the integrals are replaced with sums or optionally the free energy function is ''discretized'' by writing it in terms of δ functions being positioned at the discrete points of the state space. By applying either of these methods the upper formulas can be restated in the same form as in the (more general) continuous case, with the only exception being that functional determinants of the type, Eq. (10) (coming from integral substitutions), can be omitted.

In some standard absorber models we have the situation that the state space is mixed, i.e., it is both continuous and discrete for different state variables, and sometimes it happens that some states play a special role and have to be treated separately. Nevertheless, even in these more complicated cases it is possible to compute the absorption free energy G_{abs}, i.e., to perform a state space reduction.

To see how the reduction works in practice we will con-

sider as an example the model for the topologically driven DNA melting in (for simplicity) a homopolymeric sequence of total length L. This absorber is usually modeled [11–13] by introducing three state variables n (the number of melt base pairs), r (the number of runs of melt base pairs), and τ (the interstrand twist per melt base pair). Two of the three variables, namely n and r, are discrete and the third one τ may assume continuous values in $(-\infty,\infty)$. The free energy of the absorber is in a state $\vec{s}=(n,r,\tau)$ and the total free energy of the system absorber plasmid is given by

$$G(n,r,\tau)=\begin{cases} ar+bn+\frac{1}{2}nC\tau^2 & \text{for } n=1,2\ldots,L, \\ 0 & \text{otherwise,} \end{cases} \tag{A1}$$

$$G_{tot}(n,r,\tau,\lambda)=G(n,r,\tau)+RT\kappa\left[\lambda-\left(\frac{n}{|A|}-\frac{n\tau}{2\pi}\right)\right]^2,$$

with κ being the plasmid constant and λ the negative linking difference as introduced before,[9] a,b and C are energetic constants and $A=-10.4$ is a structural constant describing the helical winding in the B state (for details see [13]). The partition function is then given by

$$Q(\lambda)=e^{-\kappa\lambda^2}+\sum_{n=1}^{L}\sum_{r=1}^{\min(L-n,n)}\int_{-\infty}^{\infty}M(n,r) \times e^{-(1/RT)[ar+bn+(nC/2)\tau^2]}e^{-\kappa\{\lambda-[(n/|A|)-(n/2\pi)\tau]\}^2}d\tau, \tag{A2}$$

with $M(n,r)=L/r\binom{n-1}{r-1}\binom{L-n-1}{r-1}$ being the number of realizations of a state with n melt base pairs being in r runs. As we see from the occurrence of $M(n,r)$, this three variable model must already be the reduced form of a model with a higher dimensional state space. Indeed, a more detailed description within an $(L+1)$-dimensional state space $(b_1,\ldots,b_L,\tau)$ with binary variables $b_i\in\{1,0\}$ (ith base pair melt or not) and a continuous τ reduces due to the translational symmetry (sequence homopolymeric) to the three dimensional state space (n,r,τ). To perform a further reduction to only a single variable, the total negative sequence unwinding u, we simply substitute $n/|A|-n\tau/2\pi\rightarrow u$ and exchange the order of summation and integration

$$Q(\lambda)=e^{-\kappa\lambda^2}+\int_{-\infty}^{\infty}e^{-\kappa(\lambda-u)^2} \times\left(2\pi\sum_{n=1}^{L}\sum_{r=1}^{\min(L-n,n)}\frac{M(n,r)}{n} \times e^{-(1/RT)\{ar+bn+2nC\pi^2[1/|A|-(1/n)u]^2\}}\right)du. \tag{A3}$$

[9]Our λ has the opposite sign as the equivalent variable (denoted with α) in [13].

The first term on the right-hand side (that represents the weight for the ground state with 0 base pairs melt) can be brought under the integral by introducing the δ function so that finally the partition function reduces to

$$Q(\lambda)=\int_{-\infty}^{\infty}e^{-\kappa(\lambda-u)^2}e^{-(1/RT)G_{abs}(u)}du \tag{A4}$$

with

$$G_{abs}(u)=-RT\ln\left(\delta(u)+2\pi\sum_{n=1}^{L}\sum_{r=1}^{\min(L-n,n)}\frac{M(n,r)}{n} \times e^{-(1/RT)\{ar+bn+2nC\pi^2[1/|A|-(1/n)u]^2\}}\right). \tag{A5}$$

Thus we have rewritten the partition function Eq. (A2) in the reduced form by computing its absorption free energy G_{abs} as a function of the negative topological unwinding u. The unusual form of G_{abs} containing a δ function has to be attributed to the unusual structure of the underlying state space (n,r,τ) in which there are several realizations of the state $u=0$—the standard B-DNA (discrete) ground state (represented by δ) and the open-stranded but topologically relaxed states with an interstrand winding per base pair $\tau=2\pi/|A|$ that exactly compensates the denaturation unwinding and ''topologically simulates'' B-DNA. As can be seen by direct computation (or a limiting process) the occurrence of the δ function in Eq. (A5) does not affect the validity of basic identities, Eqs. (11), (17), which are implying Eq. (29). The latter in our case is written as

$$\left(\frac{1}{|A|}\frac{\partial\langle n\rangle}{\partial\lambda}-\frac{1}{2\pi}\frac{\partial\langle n\tau\rangle}{\partial\lambda}\right)>0. \tag{A6}$$

In [11] and [13] the mean value $\langle n\rangle$ for positive and negative values of λ have been obtained and they show an interesting behavior. The early study [11] has demonstrated that below the melting temperature T_m for $\lambda<0$ (positive supercoiling) $\partial\langle n\rangle/\partial\lambda<0$ holds and for $\lambda>0$ (negative supercoiling) we have $\partial\langle n\rangle/\partial\lambda>0$. These two observations, especially that for $\lambda<0$ seem to contradict the general Eq. (29). But looking more closely at Eq. (A6) this seeming contradiction quickly resolves, as Eq. (A6) states that negative values of $\partial\langle n\rangle/\partial\lambda$ are always compensated by negative values of $\partial\langle n\tau\rangle/\partial\lambda$ so that a reduction in $\langle n\rangle$ (with increasing λ) never implies a decreasing total absorption α.

On the other hand, in the later study [13] it was shown that above T_m the same absorber behaves inversely with respect to $\partial\langle n\rangle/\partial\lambda$ for decreasing $\lambda<0$ (positive supercoiling), i.e., we have there $\partial\langle n\rangle/\partial\lambda>0$. But this ''symmetric'' behavior of the transitional process below and above T_m reflected in an approximate sign inversion $\partial\langle n\rangle_{T<T_m}/\partial\lambda\rightarrow-\partial\langle n\rangle_{T>T_m}/\partial\lambda$ is imperfect and cannot hold for the λ derivative of the second structural mode $\langle n\tau\rangle$ too, as Eq. (A6) shows.

APPENDIX B

Here we will show that the conditional absorptions $@_i$ in a system of two or more topologically coupled absorbers can be computed just from the knowledge of the corresponding absorption free energies $G_{abs,i}$. The latter are linked to the unconditional absorptions α_i as given in Eq. (14) so the set of unconditional absorptions α_i determines the interaction behavior of the underlying absorbers.

For notational simplicity we restrict ourselves to the case of two interacting absorbers $\mathcal{A}_1$ and $\mathcal{A}_2$, but all our computations remain valid in the case of several absorbers. Let us suppose that the two absorbers $\mathcal{A}_1$ and $\mathcal{A}_2$ can assume a number of different conformational states described by vectorial state variables $\vec{r}=(r_1,\ldots,r_l)$ and $\vec{s}=(s_1,\ldots,s_m)$ that run through the state spaces R and S, respectively. Each conformational state of the two absorbers has the conformational free energies $G_1(\vec{r})$ and $G_2(\vec{s})$ contained in the absorbers $\mathcal{A}_1$ and $\mathcal{A}_2$, respectively. The total free energy of this two absorber system placed in a topologically closed plasmid with plasmid constant κ and with negative linking difference λ is then given by

$$G_1(\vec{r})+G_2(\vec{s})+\kappa RT\{\lambda-[U(\vec{r})+V(\vec{s})]\}^2, \qquad \text{(B1)}$$

with U and V being the topological unwinding functions of $\mathcal{A}_1$ and $\mathcal{A}_2$ respectively. Therefore the partition function Q of that composed system is given by

$$Q(\lambda)=\int_{\vec{r}\in R}\int_{\vec{s}\in S} e^{-(1/RT)[G_1(\vec{r})+G_2(\vec{s})]} \times e^{-\kappa\{\lambda-[U(\vec{r})+V(\vec{s})]\}^2} \times dr_1\ldots dr_l ds_1\ldots ds_m. \qquad \text{(B2)}$$

By applying the substitution

$$(r_1,r_2,\ldots,r_l,s_1,s_2,\ldots,s_m) \rightarrow (u=U(\vec{r}),r_2,\ldots r_l,v=V(\vec{s}),s_2,\ldots,s_m), \qquad \text{(B3)}$$

$$\left|\frac{\partial(r_1,r_2,\ldots,r_l,s_1,s_2,\ldots,s_m)}{\partial(u_1,r_2,\ldots r_l,u_2,s_2,\ldots,s_m)}\right| = \frac{1}{\left|\frac{\partial U}{\partial r_1}\frac{\partial V}{\partial s_1}\right|},$$

the upper expression can be transformed to

$$\begin{aligned} Q(\lambda) &= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{\vec{r}\in R_u}\int_{\vec{s}\in S_v}\frac{1}{\left|\frac{\partial U(\vec{r})}{\partial r_1}\frac{\partial V(\vec{s})}{\partial s_1}\right|} \\ &\quad\times e^{-(1/RT)[G_1(\vec{r})+G_2(\vec{s})]}e^{-\kappa[\lambda-(u+v)]^2} \\ &\quad\times dr_2\ldots dr_l ds_2\ldots ds_m du\,dv \\ &= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} e^{-\kappa[\lambda-(u+v)]^2} \\ &\quad\times\left(\int_{\vec{r}\in R_u}\frac{e^{-(1/RT)G_1(\vec{r})}}{\left|\frac{\partial U(\vec{r})}{\partial r_1}\right|}dr_2\ldots dr_l\right) \\ &\quad\times\left(\int_{\vec{s}\in S_v}\frac{e^{-(1/RT)G_2(\vec{s})}}{\left|\frac{\partial V(\vec{s})}{\partial s_1}\right|}ds_2\ldots ds_m\right)du\,dv \\ &= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} e^{-\kappa[\lambda-(u+v)]^2}e^{-(1/RT)G_{abs,1}(u)} \\ &\quad\times e^{-(1/RT)G_{abs,2}(v)}du\,dv, \end{aligned} \qquad \text{(B4)}$$

with $R_u:=\{\vec{r}\in R \text{ with } U(\vec{r})=u\}$ and $S_v:=\{\vec{s}\in S \text{ with } V(\vec{s})=v\}$ being the surfaces of constant unwinding of $\mathcal{A}_1$ and $\mathcal{A}_2$, respectively. The transition from the second to the third line is performed simply by applying the definition of the absorption free energy from Eq. (10).

By applying exactly the same transformation as above [Eq. (B3)], we see that the same computation also holds for

$$\int_{\vec{r}\in R}\int_{\vec{s}\in S} U(\vec{r})e^{-(1/RT)[G_1(\vec{r})+G_2(\vec{s})]}e^{-\kappa\{\lambda-[U(\vec{r})+V(\vec{s})]\}^2}dr_1\ldots dr_l\,ds_1\ldots ds_m$$

$$=\ldots=\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} ue^{-\kappa[\lambda-(u+v)]^2}e^{-(1/RT)G_{abs,1}(u)}e^{-(1/RT)G_{abs,2}(v)}du\,dv. \qquad \text{(B5)}$$

From Eqs. (B4) and (B5) we receive finally the conditional absorption

$$@_1(\lambda) := \frac{\int_{\vec{r}\in R}\int_{\vec{s}\in S} U(\vec{r}) e^{-(1/RT)[G_1(\vec{r})+G_2(\vec{s})]} e^{-\kappa\{\lambda-[U(\vec{r})+V(\vec{s})]\}^2} dr_1 \ldots dr_l\, ds_1 \ldots ds_m}{\int_{\vec{r}\in R}\int_{\vec{s}\in S} e^{-(1/RT)[G_1(\vec{r})+G_2(\vec{s})]} e^{-\kappa\{\lambda-[U(\vec{r})+V(\vec{s})]\}^2} dr_1 \ldots dr_l\, ds_1 \ldots ds_m}$$

$$= \frac{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} u e^{-\kappa[\lambda-(u+v)]^2} e^{-(1/RT)G_{abs,1}(u)} e^{-(1/RT)G_{abs,2}(v)} du\, dv}{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} e^{-\kappa[\lambda-(u+v)]^2} e^{-(1/RT)G_{abs,1}(u)} e^{-(1/RT)G_{abs,2}(v)} du\, dv}. \tag{B6}$$

A completely symmetrical expression for $@_2$ holds. In the case of a discrete state space modeling, the same arguments hold with the only exception being that the integrations are replaced by summations and the functional determinant in Eq. (B3) vanishes from expressions that follow. The generalization to the *n*-absorber case [Eqs. (35),(36)] is also straightforward and goes in an analogous way.

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